Kim, I., Mi, K., and Rao, H. (2004). Mol. Biol. Cell *15*, 3357–3365.

Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H.D., Mayer, T.U., and Jentsch, S. (1999). Cell *96*, 635–644.

Rape, M., Hoppe, T., Gorr, I., Kalocay, M., Richly, H., and Jentsch, S. (2001). Cell *107*, 667–677.

Richly, H., Rape, M., Braun, S., Rumpf, S., Hoege, C., and Jentsch, S. (2005). Cell *120*, this issue, 73–84.

Shcherbik, N., Kee, Y., Lyon, N., Huibregtse, J.M., and Haines, D.S. (2004). J. Biol. Chem. *279*, 53892–53898.

Verma, R., Oania, R., Graumann, J., and Deshaies, R.J. (2004). Cell *118*, 99–110.

Redoxing Calcium from the ER

Inositol 1,4,5-trisphosphate ($InsP_3$)-induced calcium release from the endoplasmic reticulum (ER) intracellular calcium store regulates cellular functions from the beginning of life at fertilization until death. In this issue of *Cell*, Mikoshiba and colleagues describe a novel mode of regulation of the $InsP_3$ receptor ($InsP_3R$) governed by ER luminal redox status, calcium, and pH (Higo et al., 2005).

The ER and its muscle equivalent, the sarcoplasmic reticulum (SR), are major sites of intracellular calcium storage. Following cellular stimulation, calcium is released from the ER/SR principally through the ryanodine receptor (RyR) and $InsP_3R$ calcium release channels. Subsequently, calcium is sequestered into the store by ATP consuming pumps known as the sarco-endoplasmic reticulum calcium ATPases (SERCAs). Thus, the dynamic equilibrium of these two processes shapes calcium signals in amplitude, space, and time (Berridge et al., 2003). Whereas $InsP_3Rs$ are regulated in a bell-shaped manner by cytosolic calcium and $InsP_3$, RyRs can be activated by increases in cytosolic calcium alone. Channel activity is also subject to regulation by cytosolic nucleotides, redox state, posttranslational modification, and binding of accessory proteins (Berridge et al., 2003). For example, binding of the Bcl-2 protooncogene to $InsP_3Rs$ inhibits channel opening protecting the cell from death, whereas binding of cytochrome C, which is released from mitochondria during apoptosis, inhibits $InsP_3R$ inactivation, promoting apoptosis (Hanson et al., 2004).

The positive correlation between the state of filling of the calcium store and the quantity of calcium released suggests that these channels are also subject to regulation from the ER/SR lumen. This regulatory mechanism would thus serve to either promote or inhibit calcium release from full or empty stores, thereby preventing their complete emptying and resulting pathophysiological consequences. Thus, research has been focused on identifying the lumenal calcium sensor for both of these channels. Although calcium binding sites have been identified on the lumenal face of the $InsP_3R$ and RyR, whether these sites are functional remains contentious (Sienaert et al., 1996; Ching et al., 2000). Alternatively, it has been proposed that the calcium sensor is located on an accessory protein(s), which functionally interacts with the channel. Prime candidates for this role are low-affinity calcium storage proteins such as the ubiquitous ER calcium storage protein/lectin chaperone calreticulin and the muscle-specific calsequestrin. For calsequestrin, this appears to be the case and together with its interacting partners, junctin and triadin, mediate calcium-dependent regulation of RyR opening. In this model, at low lumenal calcium, calsequestrin is stabilized in a tight complex with RyRs by triadin and junctin, inhibiting channel opening. Increasing calcium results in a weakening of the complex and channel opening is favored (Gyorke et al., 2004). The mechanism by which $InsP_3Rs$ are regulated from the lumen, however, is less clearly defined. Calreticulin has been shown to bind to $InsP_3Rs$ in a glycosylation-dependent manner, although the function of this interaction was proposed to promote the correct folding of the channel and not to regulate channel activity (Joseph et al., 1999). Binding of the ER and secretory granule-localized, low-affinity, high-capacity calcium binding proteins chromogranin A and B to $InsP_3Rs$ results in increased open probability and mean open time of channels. Since chromogranin expression is dynamic, it is likely that it may simultaneously regulate intracellular calcium storage capacity and $InsP_3R$ activity (Choe et al., 2004). Due to the limited tissue distribution of chromogranin expression, whether it acts as a general regulator of $InsP_3R$ function remains to be determined.

In this issue of *Cell*, using a proteomic approach, Mikoshiba and colleagues identify a novel $InsP_3R$ accessory protein that confers upon the receptor calcium, pH, and redox sensitivity (Higo et al., 2005). In this study, a polypeptide fragment of the $InsP_3R$ which spans its third lumenal loop (containing consensus sites for glycosylation and cysteine residues sensitive to thiol modification) was used as bait to fish for interacting partners in a brain lysate. One of the proteins isolated was identified as ERp44. Furthermore, although there is significant homology in this region between the three $InsP_3R$ isoforms, the authors determined that the interaction was specific for the type 1 $InsP_3R$. ERp44 is an ER lumenal protein that belongs to the thioredoxin protein family, members of which also include protein disulphide isomerase (PDI) and ERp57. These proteins assist in oxidative protein folding catalyzing the formation of disulphide bonds (Anelli et al., 2002). The ERp44-$InsP_3R$ interaction is dependent on the presence of cysteine residues in their reduced form in the $InsP_3R$ peptide, indicating specificity for thiol modification of the channel by ERp44. Furthermore, the interaction was sensitive to ER lumenal calcium with concentrations above 100 μM causing dissociation of ERp44 from the $InsP_3R$. This places the calcium sensitivity of the association within the physiological range of ER lumenal calcium concentration which ranges between 80 and 200 μM when full and decreasing to between 10 and 100 μM following stimulation. The functional effect of ERp44 on $InsP_3R$ activity was shown using two separate approaches. By single-cell imaging of HeLa cells, the authors demonstrated that agonist-induced calcium release was inhibited by overexpression of ERp44 and potentiated by siRNA-mediated knockdown of endogenous ERp44. No effect of ERp44 overexpression was observed on agonist-induced cal-

cium release from COS-7 cells that do not express endogenous type 1 $InsP_3R$, confirming the biochemical data. In addition, inhibition of agonist-induced calcium release was lost by mutation of the critical cysteine residues in $InsP_3Rs$ overexpressed in $InsP_3R$ knockout DT40 cells. Direct inhibition of $InsP_3R$ activity was shown by addition of recombinant ERp44 to the ER lumenal side of $InsP_3Rs$ incorporated into a lipid bilayer. Interestingly, calcium reuptake into the ER is also regulated by redox and calcium. In a recent elegant study from the Camacho laboratory, the thioredoxin ERp57 was shown to regulate SERCA2b activity (Li and Camacho, 2004). In this study, at high lumenal calcium, ERp57 and its interacting partner calreticulin associate with SERCA2b, inhibiting its activity. Store depletion results in ERp57 dissociation and relief of SERCA2b inhibition. These interactions may also be significant in the cellular response to stress, serving to protect the cell from apoptosis. Indeed, expression of both ERp57 and ERp44 is increased by cellular stress. Furthermore, ERp44 overexpression was shown in the study by Mikoshiba and colleagues to inhibit apoptosis. In conclusion, these studies underline the interdependence of oxidative protein folding and calcium signaling. In addition, they show the importance of $InsP_3$-induced calcium release in regulating apoptosis. These findings may bear great significance on the regulation of intracellular signals in cells exposed to oxidative stress such as those in tumors, following growth factor stimulation and following periods of ischemia. The relevance of these novel findings to cellular physiology will only become clear following investigation of calcium signaling in these cellular scenarios.

H. Llewelyn Roderick and Martin D. Bootman
Laboratory for Molecular Signalling
The Babraham Institute
Babraham, Cambridge, CB2 4AT
United Kingdom

Selected Reading

Anelli, T., Alessio, M., Mezghrani, A., Simmen, T., Talamo, F., Bachi, A., and Sitia, R. (2002). EMBO J. *21*, 835–844.

Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Nat. Rev. Mol. Cell Biol. *4*, 517–529.

Ching, L.L., Williams, A.J., and Sitsapesan, R. (2000). Circ. Res. *87*, 201–206.

Choe, C.U., Harrison, K.D., Grant, W., and Ehrlich, B.E. (2004). J. Biol. Chem. *279*, 35551–35556.

Gyorke, I., Hester, N., Jones, L.R., and Gyorke, S. (2004). Biophys. J. *86*, 2121–2128.

Hanson, C.J., Bootman, M.D., and Roderick, H.L. (2004). Curr. Biol. *14*, R933–R935.

Higo, T., Hattori, M., Nakamura, T., Natsume, T., Michikawa, T., and Mikoshiba, K. (2005). Cell *120*, this issue, 85–98.

Joseph, S.K., Boehning, D., Bokkala, S., Watkins, R., and Widjaja, J. (1999). Biochem. J. *342*, 153–161.

Li, Y., and Camacho, P. (2004). J. Cell Biol. *164*, 35–46.

Sienaert, I., De Smedt, H., Parys, J.B., Missiaen, L., Vanlingen, S., Sipma, H., and Casteels, R. (1996). J. Biol. Chem. *271*, 27005–27012.